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Trends in the patterns of IgM and IgG antibodies in febrile persons with suspected dengue in Barbados, an English-speaking Caribbean country, 2006—2013

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Summary Long-term seroprevalence studies of dengue have provided a measure of the degree of endemicity and future trends in disease prevalence and severity. In this study, we describe the seroprevalence of dengue antibodies in febrile persons with suspected acute dengue in Barbados. It is a retrospective population-based study of all febrile persons with suspected dengue from 2006 to 2013. All of the cases had IgM and IgG antibodies in the blood sample drawn between days 3 and 5 of their illness. Among the 8296 cases that were tested for IgM antibodies, 3037 (36.6%) had recent dengue infection. In the age groups <5 years, 5—20 years and >20 years, 23.3%, 39.6% and 35.5% had acute infection, respectively. Of the 7227 cases with documented IgG results, 5473 (75.7%) were positive and had a past infection. In the age groups <5 years, 5—20 years and >20 years, 31.2%, 65.2% and 86.6%, respectively, had a past infection (IgG positive). During the first 5 years of life, 10—20% of febrile persons investigated for dengue had a positive IgM and a negative IgG titer, between 5 and 10% had a positive IgM and IgG titer, 5% had a positive IgG and a negative IgM titer, and between 45% and 65% had a negative IgM and a negative IgG titer. Throughout the study period, between 12% and 20% of febrile persons failed to show any evidence of current or previous dengue. In the age groups <5 years, 5—20 years and >20 years, 45.0%, 18.8% and 7.2%, respectively, had no evidence of recent or past dengue (both IgM and IgG negative). Between 37% and 59% of the febrile persons had serological evidence of past dengue in the absence of any current dengue. In conclusion, the pattern of IgG antibodies in this study was comparable to those in

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countries known to be hyperendemic for dengue. The age of infection is likely to shift to younger adults and children who are more likely to have severe dengue in the future.

Introduction

Infection with any of the four serotypes of dengue (DENV 1—4) results in a spectrum of manifestations from asymptomatic to severe dengue, which can be fatal if not detected and treated early. Sequential (secondary or tertiary) infection with a different dengue serotype can, in some instances, result in a severe form of dengue, such as DHF [1—5]. Dengue has been hyperendemic in southeast Asia since the 1950s. All four serotypes circulate in the population, and there is a high dengue seroprevalence with a high incidence of DHF [6]. In Latin America and the Caribbean, dengue re-emerged as a public health problem in the 1980s, with almost all territories reporting circulation of all four serotypes by the early 1990s [7—9]. In general, the transmission of dengue (the endemicity) in this region is thought to be lower with fewer reported cases of DHF compared to the situation in southeast Asian countries. However, more recent studies from some of the countries in the Americas including the Caribbean have demonstrated that most dengue cases presenting to hospitals and health centers were secondary cases, suggesting a much higher transmission or endemicity than previously thought [10—13].

Seroepidemiological studies provide a measure of the endemicity of dengue. Additionally, they are useful in predicting epidemics of the more severe disease forms. Although a population-based seroepidemiological study would yield much more valid results, limited resources in these settings are a constraint for such studies. There are only a few short-term dengue seroepidemiological studies from the English Caribbean [12—15]. Barbados, one of the eastern Caribbean countries with a population of 284,600 in 2013 [16], has a fairly efficient primary and tertiary health care network, both in the public and the private sector, with good inter-sectorial cooperation in health care delivery. There is an active surveillance system for dengue, a detectable disease, with a centralized dengue laboratory that provides dengue testing for all Barbadians free of charge. The objective of this study was to describe the seroepidemiology of dengue in this country in a select population of febrile persons suspected and tested for dengue over an 8-year period.

Material and methods

Study design and study population

This is a retrospective study. The study population consisted of all febrile persons who presented to any of the health care facilities in Barbados and who were suspected and investigated for dengue. All age groups were included in this study. In this country, dengue is a notifiable disease, and the ministry of health has established written guidelines for suspecting and investigating dengue that are based on the guidelines from the World Health Organization. These guidelines are circulated to all doctors periodically. Febrile patients may present to the state-run primary health centers, to the offices of medical practitioners across the island, or to the emergency department of the Queen Elizabeth Hospital (QEH). If suspected to have dengue, the patient is examined for dengue by the attending physicians, including dengue IgM and IgG tests. The request form for IgM and IgG antibody testing requires detailed patient demographics, including age, gender and home address. Additionally, the forms require clinical details including date of onset of the illness, date of sampling, list of all clinical manifestations, any pre-existing chronic medical or surgical conditions, whether the person is being treated as ambulatory patient or as an inpatient at a hospital, and the site of care where the sampling was done. The attending physician filled out the dengue request form that accompanied the blood sample to the laboratory. All dengue serology tests were performed free of charge at a central dengue laboratory for the entire country. Suspected cases in which neither IgM nor IgG results were available were excluded from further analysis. The study period extended from January 2006 through December 2013. The study was approved by the Institutional Review Board for ethics in human studies at the University of the West Indies (Cave Hill) and the Ethics Committee of the QEH.

The dengue laboratory routinely maintained all demographic and clinical information entered in

the dengue test request form for all of the suspected dengue patients tested for the anti-dengue IgM and IgG antibodies in a Microsoft Access database. All of the patients' information contained in the request form and the results of the tests were entered into the database by the laboratory clerk to avoid any bias. Data for this study were extracted from the database maintained by the dengue laboratory and entered into a Microsoft Access database. Extracted data included the patient's age and gender, date of onset of the illness, date of testing, the test results, and whether the patient was cared for as an ambulatory patient or as an inpatient at a hospital. All entries for the serology test requests in suspected dengue cases were included, irrespective of the test results for the IgM or IgG antibodies. Once again, the data extraction for this study was performed by the laboratory clerk to avoid bias. Patients for whom the IgM or the IgG test results were not available were excluded from further analysis.

Methods

As a routine procedure for all suspected dengue cases, 3—5 ml of clotted blood was drawn for dengue IgM and IgG serological studies at the time of presentation to the health care facility and sent to the centralized dengue laboratory on the same day. If the initial sample was taken during the first 3 days of the illness and tested negative for IgM antibodies, a repeat sample was drawn after day 5 of the illness for repeat IgM testing. This cut-off time was established based on our preliminary findings from data analyzed for the period between 2000 and 2005, which showed that the majority of the testing was performed on day 4 of the illness and that the proportions of samples positive on days 1, 2 or 3 were significantly lower than those on day 4, day 5 and after day 5. This also showed that although the proportion of positivity was higher for samples after day five when compared to days 4 and 5, it was not statistically significant. A final report from this analysis, which includes samples through 2013, is under preparation for publication. However, not all of the persons who tested IgM negative on the first sample taken during the first 3 days of the illness had a repeat sample for IgM testing. Overall, there were 5872 persons (70.7% of the total 8296 persons tested for IgM at any time of the illness) who tested IgM negative on the first sample. Of those who tested IgM negative on the first sample, 4245 samples were taken on day 4 or later in the illness (64.9% of the 6541 samples taken on day 4 or later) and 1627 samples were taken during the first 3 days of the illness (95.3% of the 1755 samples taken during first 3 days of the illness). Out of the 1627 negative IgM samples taken during the first 3 days of the illness, 1444 (88.7%) had a repeat sample taken after day 5 of the illness for IgM testing, 831 (57.5% of those who had a repeat sample taken after day 5 of the illness) were still negative and 613 (42.5% of those who had a repeat sample taken after day 5 of the illness) seroconverted to IgM positive. The remaining 183 (11.3%) samples taken on the first 3 days of the illness and which were negative on the first tests were not retested. Serological tests were performed for the detection of dengue IgM and IgG antibodies using a commercially available Enzyme Linked Immuno-Sorbent Assay (ELISA) kit. Dengue IgM capture DxSelect ELISA and Dengue IgG DxSelect ELISA (Focus Diagnostics, Cypruss, CA) were used. The manufacturer's instructions were followed for the testing process. This commercially available assay has been evaluated and was found to be sufficiently sensitive and specific for the sero-diagnosis in clinical serum samples [17].

All of the patients were managed supportively by the attending physician using recent WHO guidelines for the management of dengue [18]. Following standard practice in this country, all cases of severe forms of dengue or those at risk for a severe form of dengue were referred to the QEH for admission and further supportive management.

Definitions

A probable [suspected] case of dengue was defined by the presence of fever with two or more of the following features: rash, nausea and vomiting, aches and pains, positive tourniquet test, leucopenia and any one of the warning signs or any of the criteria for severe dengue [18]. The presence of a positive IgM antibody in the blood was considered to confirm a recent dengue infection. Recent confirmed dengue was classified as a Primary or Secondary [sequential] case based on the absence or presence of positive IgG in the samples collected during the first 5 days of the illness from its clinical onset. Past dengue was defined by the presence of IgG titer in the blood sample collected during the first 5 days of the illness and a negative IgM titer in the blood sample collected at any time after day 4 of the illness. The symptomatic confirmed dengue infection rate was calculated by dividing the number of persons with symptomatic confirmed dengue by the total population and was expressed per thousand persons per year.

All data were stored in a password-secured Microsoft Access database to which only the authors had access. Data were analyzed using the SPSS

statistical package and tables and graphs were generated using the same software. We used the chi-square test to determine the significant difference in categorical variables. A P value of less than 0.05 was considered significant. All of the 95% confidence intervals were corrected for continuity.

Results

During the 8-year study period, there were a total of 8432 febrile persons who were suspected and investigated for dengue. The annual incidence of probable dengue ranged from 1.3/1000 population (378 cases) in 2009 to 6.8/1000 population (1931 cases) in 2013. The median duration of symptoms suggestive of dengue at the time of blood sampling was 4 days (range 2—5 days). An overview of the analysis of serology data of suspected dengue patients who were investigated during the study period is shown in Table 1. Approximately 8296 (98.4%) cases had a documented IgM result, 7227 (85.7%) cases had an IgG result, and 7091 (84.1%) suspected cases had full dengue serology (both the IgM and IgG) status.

The age of the patients with suspected dengue ranged from less than 1 year to 101 years (mean age 29.8 years, median age 26 years, SD = 21.0). Among the suspected dengue cases, there were 4599 females and 3849 males, with a male to female ratio of 0.84. The mean age for the females was 30.6 years, and the mean age for males was 28.9 years ($P = 0.0006$). A plot of the number of suspected cases against the age showed a disproportionately higher number during the first 2 years of life, followed by a sharp fall and then a gradual rise in numbers in the age range of 10—25 years, followed by a negative exponential smoothing of the graph for both males and females.

Of the 8296 cases that were tested for IgM antibodies, 3037 (36.6%; 95% CI = ±8.3%) cases tested positive and were confirmed to have recent dengue. In the age groups <5 years, 5—20 years and >20 years, 23.3%, 39.6% and 35.5% had acute infection, respectively. No significant difference was noted in the proportion of suspected cases that were positive for IgM antibodies when analyzed for males and females ($P = 0.95$) and for various age groups ($P = 0.33$). However, if we excluded the group above 85 years old (as there were fewer observations in this group), there was a significant positive correlation between the proportion of suspected cases that were IgM positive and the patients' age ($r^2 = 0.15$, $P = 0.0003$).

Of the 7227 suspected cases that had documented IgG results, 5473 (75.7%; 95% CI = ±2.57%) were positive for IgG antibodies in the sample taken during first 5 days of their illness. Fig. 1 is a plot of the proportion of suspected cases that were IgG positive against age. In the age groups <5 years, 5—20 years and >20 years, 31.2%, 65.2% and 86.6% had past infection, respectively. For the age group over 80 years ($n = 126$), 90.4% of probable dengue cases who were investigated had a positive IgG in their serum sample taken during the first 5 days of their illness. In the age groups <5 years, 5—20 years and >20 years, 45.0%, 18.8% and 7.2%, respectively, had no evidence of recent or past dengue.

Fig. 2 is a plot of the different serodiagnoses against the age of the patients. During the first 5 years of life (excluding the first year), between 10% and 20% of febrile children suspected and investigated for dengue had positive IgM titers and negative IgG titers (categorized as recent primary dengue), between 5 and 10% had positive IgM and IgG titers (categorized as recent secondary dengue), approximately 5% had positive IgG titers and negative IgM titers (past dengue), and between 45% and 65% had negative IgM and negative IgG titers (no recent or past dengue). The first year was an exception, as close to 50% of febrile children suspected and investigated for dengue had positive IgG titers with negative IgM titers. In persons over 20 years who were suspected and investigated for dengue, between 20% and 50% had positive IgM and IgG titers (secondary dengue), less than 10% had positive IgM titers and negative IgG titers (primary dengue), and 50% to 60% had negative IgM titers and positive IgG titers (past dengue). These differences in the serodiagnosis among the different age groups were statistically significant ($P = 0.01$).

An analysis of the time trends in the serodiagnosis over the study period is shown in Fig. 3. The years 2006, 2008, 2009, 2010 and 2011 were considered non-epidemic years, whereas the years 2007, 2012 and 2013 were epidemic years. During the 8-year study period, an overall 16.1% (95% CI = 15.2%, 17.0%) of febrile persons suspected and investigated for dengue failed to show any evidence of current or previous dengue (negative IgM and IgG titers), and this proportion ranged between 13.1% (2013) and 20.1% (2009). No significant time trend was noted. Overall, 8.1% (95% CI = 7.5%, 8.8%) had recent primary infection and 28.8% (95% CI = 27.8%, 29.9%) had secondary infection. During the non-epidemic years, of the febrile persons suspected and screened for dengue, 4.9% (95% CI = 4.1%, 5.8%) had recent primary infection and 23.4% (95% CI = 21.8%, 25.1%) had secondary infection. The corresponding figures for the

Table 1 Summary of the immunoglobulin findings among febrile persons with suspected dengue in Barbados, 2006—2013.

	Dengue IgM		Dengue IgG		*N*	N_{total}
	Negative	Positive	Negative	Positive		
Incomplete Ig status						
Recent dengue? — no previous dengue			38		38	
Recent dengue? — previous dengue				98	98	
No dengue — previous?	787				787	
Recent dengue — previous?		418			418	
Total						1341
Complete Ig status						
No dengue — no previous dengue	1141		1141		1141	
No dengue — previous dengue	3331			3331	3331	
Recent dengue — no previous dengue		575	575		575	
Recent dengue — previous dengue		2044		2044	2044	
Total						7091
Totals		5259	3037	1754	5473	8432
		Total IgMs	8296	Total IgGs	7227	

epidemic years were 10.0% (95% CI = 9.2%, 10.9%) and 32.0% (95% CI = 30.7%, 33.4%). The difference in the proportion of primary and secondary cases during the non-epidemic and epidemic years was significant ($P < 0.003$). Overall, 47.0% (95% CI = 45.8%, 48.2%) of those tested for dengue had evidence of past infection without any evidence of recent infection. In the non-epidemic and epidemic years, 55.2% (95% CI = 53.3%, 57.1%) and 42.1% (95% CI = 40.7%, 43.6%), respectively, had past infection in the absence of recent infection. The difference between the epidemic and non-epidemic years

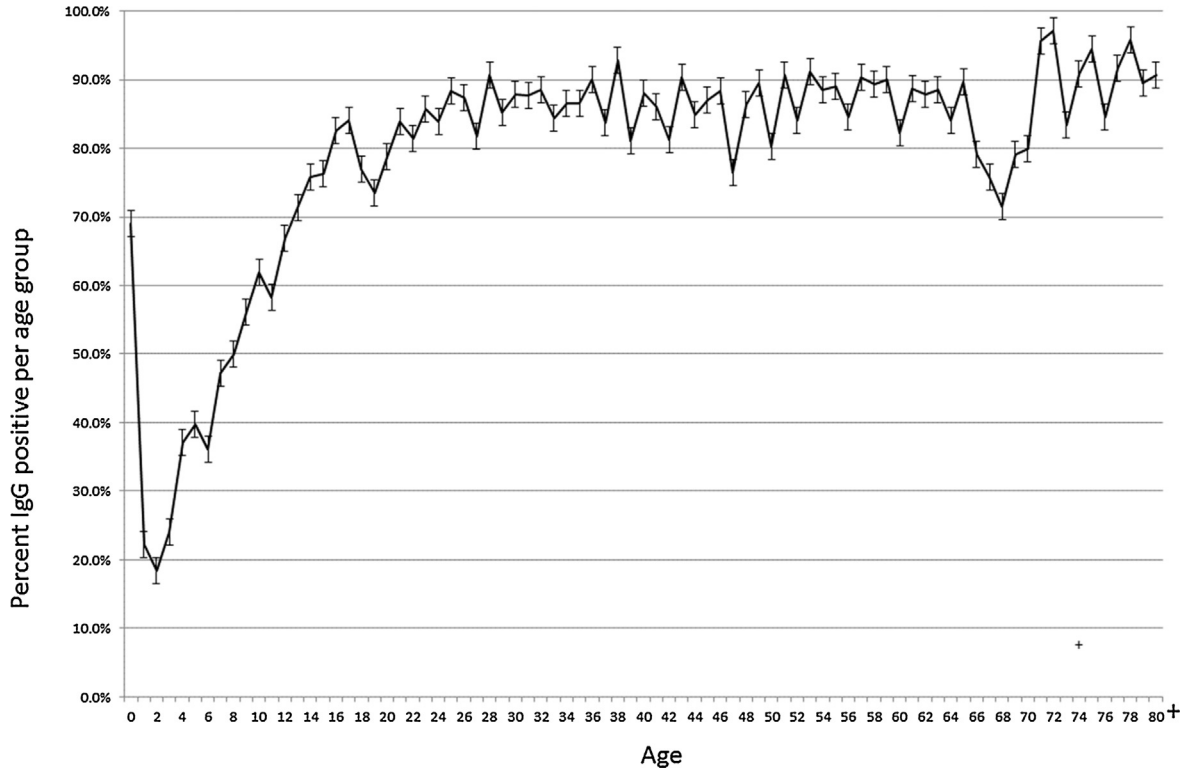

Figure 1 Dengue IgG seroprevalence by age in Barbados, 2006—2013.

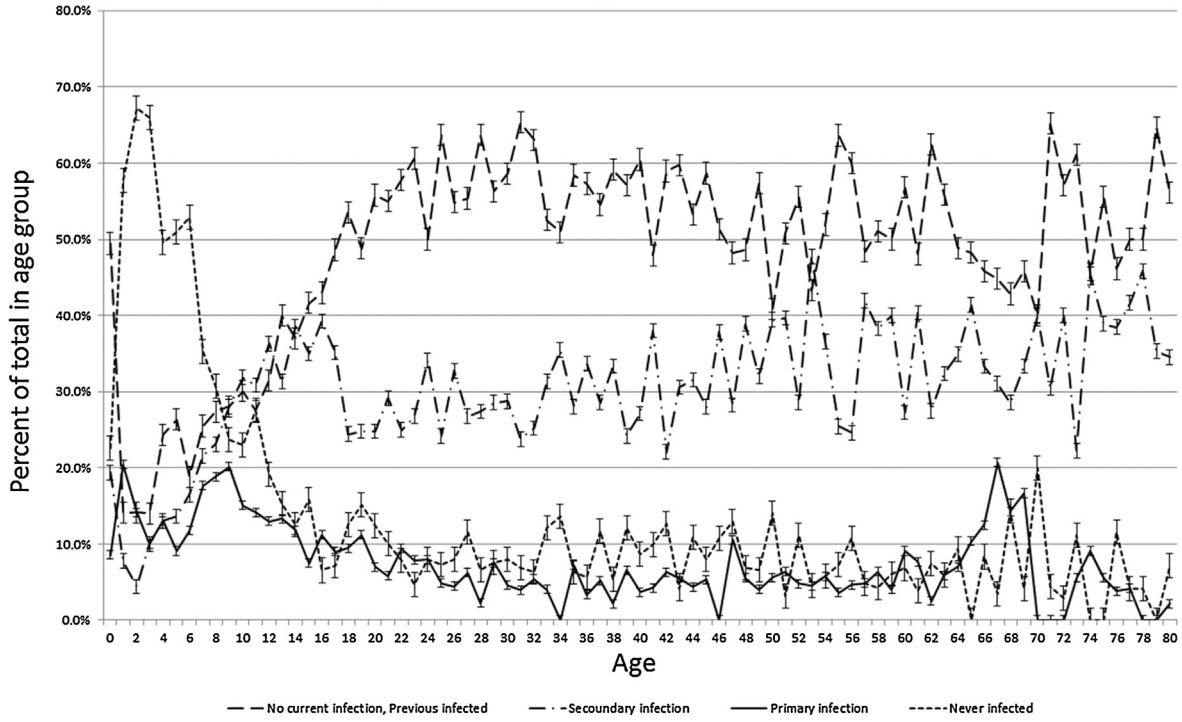

Figure 2 Serological diagnosis by age group among febrile persons with suspected dengue in Barbados, 2006—2013.

was significant ($P < 0.0001$). Over the study period, 37.0% (213/575; 95% CI 30.95%, 39.94%) and 37.9% (774/2044; 95% CI = 34.64%, 39.24%) of all confirmed recent primary and secondary (sequential) cases, respectively, were among the hospitalized persons.

Discussion

This study of the pattern of IgM and IgG responses among the febrile persons who were suspected to have dengue and presented to the various health care facilities throughout Barbados is the

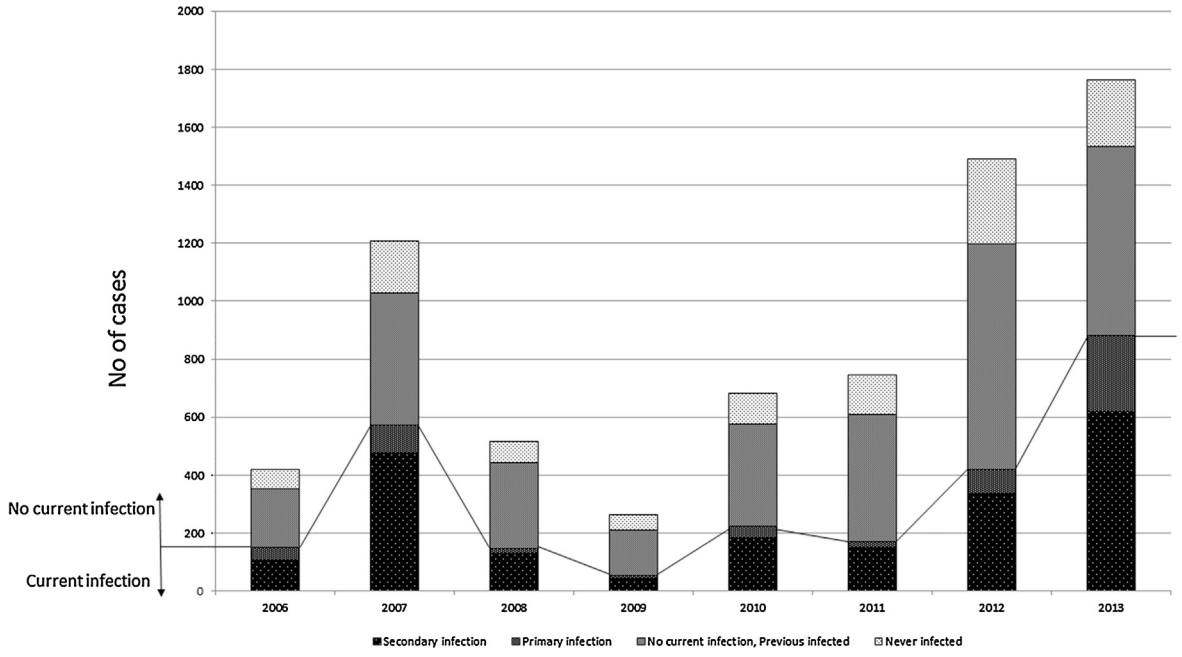

Figure 3 Time trend in serological diagnosis among febrile persons with suspected dengue in Barbados, 2006—2013.

first long-term seroepidemiologic study reported from an English-speaking Caribbean country. The age-specific seroprevalence findings and the time trend in the seroprevalence in this select population provide useful insight into the dynamics of dengue transmission in this population. These findings should provide useful predictions of the future course and the nature of dengue epidemics in this country and in the Caribbean region as a whole.

In Barbados, in the eighties and early nineties when dengue re-emerged in the Caribbean, only DENV 2 was reported, which was responsible for sporadic cases. However, by 1995, DENV 1, DENV 2 and DENV 4 were in circulation in this country and in the wider English Caribbean. In this region, dengue became a major public health problem in the mid-nineties, with two major epidemics in 1995 and 1997 [19,20]. DENV 3 reemerged in the English Caribbean in 1997 and by 2002 had spread to most of the Caribbean [20]. In 2001, all four serotypes were co-circulating in this country, and there was an epidemic in 2002, which was a part of the pan-Caribbean pandemic [13,20]. In the time period between 2003 and 2007, DENV 3 caused almost all cases of dengue in this country [21]. Barbados, one of the popular tourist destinations in the Eastern Caribbean, has a tropical climate with high annual rainfall and humidity. This climate is conducive to the propagation of the *Aedes aegypti* mosquito and results in a high population density, which provides favorable conditions for dengue transmission.

The findings from this study show that by the age of 18, over 80% of the studied population has seroconverted and by 25 years, more than 90% of the study population was positive for the IgG antibodies to one or more dengue serotypes (Fig. 1). There was a dip noted in the IgG seroprevalence in the age group from 65 to 75 years. This may be reflective of the lesser likelihood of the persons in this age group to present for care and be examined for dengue. In Barbados, the age of retirement is 65 years, and thereafter, there is no need for the mandatory health certification after illness that applies to working people. However, with further advancing age beyond 75 years, health issues are taken more seriously, and individuals are more likely to seek care for suspected dengue; therefore, the seroprevalence returned to the higher levels seen in age groups younger than 65. Nearly 100% of persons in their eighties and older were positive for the IgG antibodies. A similar pattern of high seroprevalence of IgG was reported in a short-term seroprevalence study of the sentinel population from two other neighboring Caribbean countries [12,14]. The pattern of seroconversion seen among a select population of children attending the medical facility for gastrointestinal symptoms in the Dominican Republic [12] and among febrile persons who were screened for dengue in Jamaica [14] was similar to the pattern seen in this study. Interestingly, the pattern of IgG seroprevalence seen in our study was also similar to the patterns of high IgG seroprevalence that were reported in population-based prospective studies from some of the other highly endemic countries in the Americas [11,22—25] and those of other highly endemic countries in southeast Asia [26—28].

The rate of seroconversion over a period of time is a rough indicator of the intensity of transmission of the virus in the population [29,30]. In the age groups <5 years, 5—20 years and >20 years, 31.2%, 65.2% and 86.6%, respectively, had past infection (IgG positive). This compares well with the figures reported from Jamaica, another English-speaking Caribbean country [14]. In this study population, at least 20% of children were found to have already had dengue infection and seroconverted by their second birthday; this would translate to a seroconversion rate of 10% per year in the first few years of life (Fig. 1). However, some of these IgG-positive infants may have had trans-placental transfer of IgG from their mother. Nevertheless, this may be reflective of a fairly high rate of transmission of the virus and a high vector density in this population. A high rate of seroconversion with age with a lower mean age of infection seen in this population could be interpreted as a measure of the intensity of the endemicity of this infection in this population [29—31]. The pattern of high IgG seroprevalence seen in this population in the presence of the co-circulation of all 4 dengue serotypes is in keeping with the high proportion of secondary infections seen in this study, especially among young adults.

In the age groups <5 years, 5—20 years and >20 years, 23.3%, 39.6% and 35.5% had acute infection (IgM and/or NS1 positive), respectively. These figures are higher than those reported from another seroprevalence study from this region [14]. Less than 10% of the adults in this study population (febrile persons with suspected dengue) had evidence of recent infection that was categorized as primary (IgG negative and IgM positive in the acute serum sample), whereas between 30 and 40% of febrile adults with suspected dengue had secondary infection (Fig. 2). In other words, current dengue was three to four times more likely to be a secondary than a primary infection. A similarly higher proportion of secondary infection has been reported from other countries

known to be hyperendemic for dengue [23,27,32]. A continuing high incidence of recent infection in adults in the presence of high IgG seroprevalence would mean that this population is immune to only some of the dengue serotypes. Therefore, further sequential dengue infection with heterologous serotypes in partially immune individuals would potentially increase the risk of more severe forms of dengue such as DHF in the future [1,2,5,33,34].

A notable observation in this study was very high IgG seropositivity among the older adults over the entire period of this study in the face of co-circulation of all four serotypes of dengue and continuing acute infection in this population. There are at least three important inferences that could be drawn from this observation. First, the older adults in this population should be nearing their saturation point of immunity to multiple serotypes of dengue. Thus, older adults should have a very low susceptibility to further acute infection with any of the dengue serotypes in the future [30]. This is already reflected in the very low numbers of acute infection in the age group older than 80 in this study (Fig. 3). Second, in the face of continuing circulation of multiple dengue serotypes, the age of infection is bound to shift to the lower age group in the coming years in this population. This is a phenomenon that is very similar to those experienced in many of the highly endemic countries of southeast Asia and the Americas [11,31,35]. Third, this would mean that the risk of severe dengue from sequential infection should have already reached a plateau and that there may not be further increases in the proportion of severe forms of dengue in this population in the future.

One of the major limitations of this study was the lack of data on the serotype-specific antibody identification due to the lack of testing facilities. As a result, we were not able to detect the multiplicity of previous infections or ascertain the sequence of past infection and its relationship with disease severity in this population. The unavailability of data on the total number of all febrile persons seen over the study period makes it difficult to assess the degree of completeness of the screening process, and it is likely that this study may have underestimated the actual prevalence of dengue infection in this population. The retrospective nature of this study is another limitation of this study. The retrospective nature of the study was also responsible for the significant amount of missing data. We could not subject the findings from this study to any external validation due to the lack of similar types of data from this population or from the other English Caribbean countries in this region.

In summary, the results of this sentinel study from the English Caribbean show that the seroprevalence of dengue-specific IgG in this representative sample of the population is very high and stable over the fairly long study period. The near-saturated seroprevalence of IgG seen in this population of febrile adult patients could translate into increased infection among younger children in contrast to the present scenario in which the majority of dengue cases in the population occur in younger adults. Additionally, in the future, dengue is likely to present with more severe disease patterns in younger adults and children who have a significantly high IgG level but are not yet at saturation levels, making them prone to more severe disease patterns associated with sequential infections. Therefore, this study encourages the vector control and epidemic containment as well as the formulation of standard local guidelines for the early detection and optimal management of dengue in this country. However, there is a need to further explore the serotype-specific seroprevalence, which is useful in the early prediction of epidemics.

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Conflicts of interest

None declared.

Authors' contributions

Alok Kumar: Study design, data collection and writing of the manuscript.

Anders L. Nielsen: Data validation, data analysis, tabulation and review of the manuscript.

Ethical approval

Ethical approval was obtained from the Institutional Review Board on Ethics in Human Studies at the University of the West Indies [Cave Hill], Barbados.

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